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Two new species of *Rhytismataceae* on fagaceous trees from Anhui, China

Li Chen[1], D.W. Minter[2], Shi-Juan Wang[1] & Ying-Ren Lin[1*]

[1] *School of Forestry & Landscape Architecture, Anhui Agricultural University, West Changjiang Road 130, Hefei, Anhui 230036, China*

[2] *CAB International, Bakeham Lane, Egham, Surrey, TW20 9TY, UK*

*Correspondence to: *yingrenlin@yahoo.com*

Abstract — *Lophodermium circinatum* sp. nov. on *Castanopsis eyrei* and *Terriera nitens* sp. nov. on *Cyclobalanopsis myrsinifolia* and *C. pentacycla* from China are described, illustrated, and discussed. Type specimens are deposited in the Reference Collection of Forest Fungi of Anhui Agricultural University, China (AAUF).

Key words — conservation, foliicolous fungi, morphology, parasitism, taxonomy

Introduction

During fieldwork in China studying leaf-inhabiting fungi on members of the *Fagaceae*, three specimens of the ascomycete family *Rhytismataceae* were collected. Examination of that material and comparison with species already described led to the conclusion that one specimen represents a new species of *Lophodermium* Chevall. and the other two represent a new species of *Terriera* B. Erikss.

Materials & methods

For microscopic examination, pieces of leaf material with fruitbodies were selected from each collection. The external appearance of the fungal colonies, zone lines, ascomata, and conidiomata were observed with a dissecting microscope at 10–50× magnification. After 10–15 min. in water, fruitbodies were cut into 10–15 μm thick vertical transverse sections using a freezing microtome (YD-202, China). These were mounted in lactic acid or cotton blue (with pretreatment in water) onto microscope slides, and examined. Squash mounts in water, lactic acid or cotton blue were made to observe asci, ascospores and paraphyses. Gelatinous sheaths surrounding ascospores and paraphyses were examined in water or 0.1% (w/v) cotton blue in lactic acid. The colour of internal structures and ascus contents were observed in water. Measurements were made from more than 30 asci, ascospores, paraphyses, conidiogenous cells and

conidia for each specimen using material mounted in 5% KOH or Melzer's reagent. Line and point integrated drawings of external shapes and internal structures of fruitbodies were prepared using a microscope drawing tube (Panasoianic XSJ-2, Japan).

Taxonomy

Lophodermium circinatum Li Chen bis & Y.R. Lin, **sp. nov.** Figs 1–7

Fungal Name FN570066

Differs from *Lophodermium yangii* by subcuticular ascomata, well-developed lips, much shorter asci and ascospores, and paraphyses with circinate apices.

Type: China, Anhui, Qingliangfeng, Zhujiashe, alt. 180 m, on fallen leaves of *Castanopsis eyrei* (Champ. ex Benth.) Hutch. (*Fagaceae*), 4 July 2006, T. Zhang & Y.R. Lin 2129 (**Holotype** AAUF 68937).

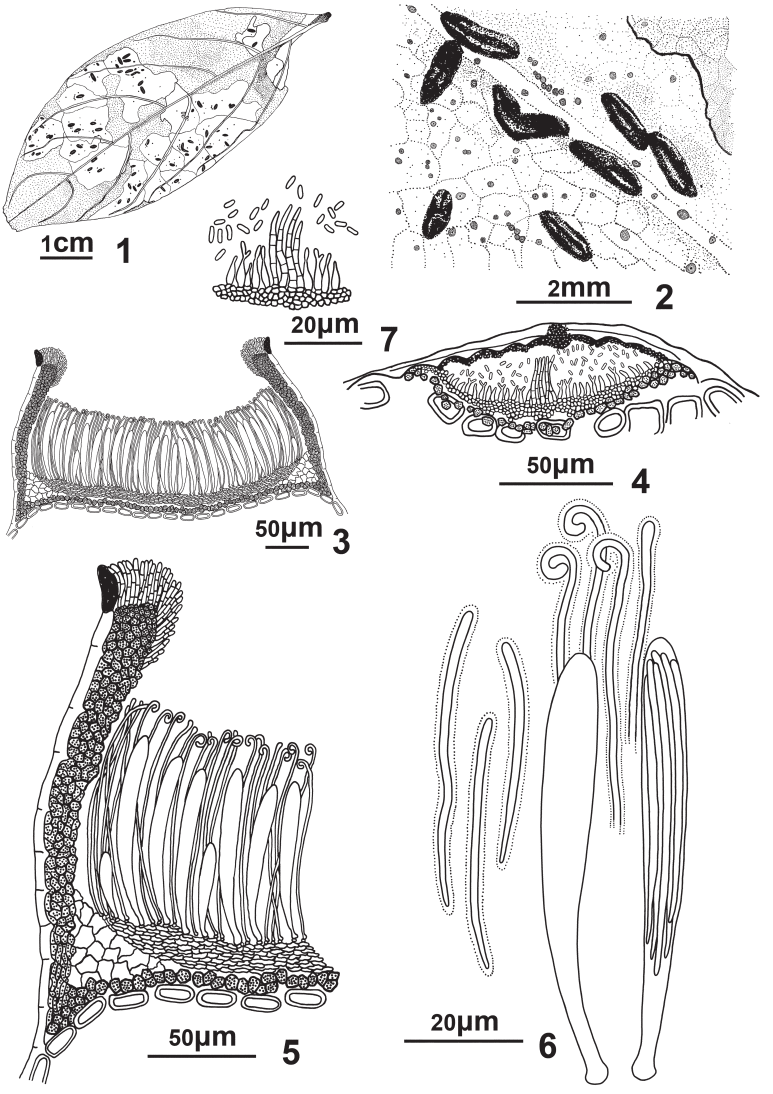
Etymology: *circinatum*, referring to the paraphyses, which are circinate at the apex.

Colonies on both sides of leaves, forming subcircular or irregular, light yellow to grayish-yellow spots, 10–18 mm diam., which tend to coalesce into larger irregular shapes.

Zone lines frequent, gray-black and thin, entirely or partly surrounding the paler areas, not always conspicuous.

Conidiomata on both sides of leaves, predominantly on the upper side, crowded, sometimes with several coalescing. In surface view, conidiomata 90–175 × 80–135 μm, almost circular to elliptical, slightly raising the substratum surface, concolorous with the substratum surface or light yellow-brown, dark-brown in the centre and at the perimeter line, often universally brown after spore discharge which occurs through an apical ostiole. In vertical section, conidiomata subcuticular. Upper wall 3–6 μm thick, blackish brown, composed of tiny angular cells. Basal wall poorly developed, brown to dark brown, consisting of 1–2 layers of angular and globose, thick-walled cells 2.5–4.5 μm diam. Subconidiogenous layer 3.5–8 μm thick, consisting of colourless, thin-walled angular cells. Trichogynes 20–28 × 2–3 μm, cylindrical, tapering towards the rounded apex, 4–6-septate. Conidiogenous cells 8–12 × 2–2.5 μm, flask-shape, holoblastic, proliferating sympodially. Conidia colourless, aseptate, smooth, cylindrical or elliptical, 3–4.5 × ca 1 μm.

Ascomata in similar positions to conidiomata on the substratum, scattered, occasionally with 2 coalescing in the paler areas. In surface view, ascomata 900–1750 × 360–650 μm, elliptical to elongate-elliptical, straight or curved to one side, ends obtuse or slightly acute, black, shiny, with a clearly marked outline, moderately raising the substratum surface but clearly sunken near the split, opening by a single longitudinal split which extends almost to the ascomatal edge. Lips present. In median vertical section, ascomata subcuticular. Covering stroma 20–28 μm thick near the opening, slightly thinning towards the edge, connecting to the basal stroma, composed mainly of textura angularis



Figs 1–7. *Lophodermium circinatum* on *Castanopsis eyrei*. 1. A leaf bearing zone lines and fruitbodies. 2. A zone line and fruitbodies observed under a dissecting microscope. 3. Ascoma in median vertical section. 4. Conidioma in vertical section. 5. Portion of ascoma in median vertical section. 6. Paraphyses, asci, and ascospores. 7. Trichogynes, conidiogenous cells, and conidia.

with dark brown to blackish brown, thick-walled cells 3–5.5 µm diam. Lip cells well developed, arranged somewhat radially, 10–24 × 1.5–2.5 µm, cylindrical, the apical cells sometimes slightly wider, nearly colourless, with black and markedly brittle tissue sticking to the outer side. Basal stroma dark brown, composed of 1–2 layers of thick-walled, angular to globose cells 4–6 µm diam. The triangular area, visible in sections between the covering stroma and the basal stroma at each edge of the ascoma, is filled with colourless, gelatinous, thin-walled angular cells 4–9.5 µm diam. Subhymenium 10–16 µm thick, rather flat, consisting of colourless textura porrecta. Paraphyses 90–120 × 1.2–1.5 µm, filiform, usually curved into uncinate or circinate shapes, sometimes gradually widening to 2–2.5 µm at the apex, and immersed in a ca 1.5 µm thick gelatinous matrix. Asci ripening sequentially, 70–95 × 7–9 µm, cylindrical-clavate to cylindrical, somewhat long-stalked, thin-walled, apex rounded, without circumapical thickening, J–, 8-spored. Ascospores arranged in a fascicle, 40–55 × 1.2–1.6 µm, filiform, colourless, aseptate, slightly tapered towards the rounded base, and immersed in a gelatinous sheath ca 1 µm thick.

Associated organism, habitat, and distribution: *Castanopsis eyrei*, on leaves. Known only from the type locality, Anhui Province, China.

Comments—*Lophodermium* is the largest genus of the *Rhytismataceae*. IndexFungorum (2013) lists 282 specific epithets for the genus, of which SpeciesFungorum (2013) accepts 135. Although exceptions exist, in the *Rhytismataceae* individual species tend to occur on only conifers, only dicotyledons, or only monocotyledons, while species on dicotyledons tend to occur on either herbs or woody plants but not both. These patterns help limit the taxa for comparison when assessing possible new species.

No *Lophodermium* has been described with type material on *Castanopsis*, but 14 species from IndexFungorum have been recorded on trees of the *Fagaceae* (Cannon & Minter 1986; Farr & Rossman 2013; Johnston 1989a, b, 2001; Lin et al. 2002). *Lophodermium echinophilum* Speg., *L. gamundiae* P.R. Johnst., *L. mahuianum* P.R. Johnst., *L. medium* P.R. Johnst., *L. petiolicola* Fuckel, *L. punctiforme* (Fr.) Fuckel, *L. quercus* S.K. Bose & E. Müll., and *L. yangii* Y.R. Lin & C.L. Hou have type material on other fagaceous genera and are known mainly in association with the *Fagaceae*. *Lophodermium agathidis* Minter & Hettige, *L. eucalypti* (Rodway) P.R. Johnst., *L. foliicola* (Fr.) P.F. Cannon & Minter, *L. maculare* (Fr.) De Not., *L. mangatepopense* P.R. Johnst., and *L. minus* (Tehon) P.R. Johnst. were originally described from leaves of other woody plants but have subsequently also been recorded in association with the *Fagaceae*.

None of the *Lophodermium* species with type material on the *Fagaceae* have paraphyses with circinate apices. *Lophodermium gamundiae*, *L. mahuianum,* and *L. medium* were all described on *Nothofagus* species from New Zealand or southern South America. No anamorph or zone lines have been observed for

L. gamundiae, and lengths of asci (160–190 µm) and ascospores (60–80 µm) are much greater (Johnston & Park 2007) than for our new species. Johnston (1989b) described *L. mahuianum* as never having zone lines or an anamorph, and lengths of asci (130–175 µm) are also much greater. Ascomata of *L. medium* are often triangular in outline and its asci (100–140 µm; Johnston 1989b) are longer than those of *L. circinatum*. Recent evidence suggests that *Nothofagus* should be placed in its own family separate from the *Fagaceae* and the natural distribution of *Nothofagus* in South America and Australasia apparently represents a single population divided when continental drift made Antarctica too cold for plants. Similar distributions might be expected for species of the *Rhytismataceae* evolving with *Nothofagus*. The present fungus is thus clearly different from these three species.

Their protologues described both *L. petiolicola* and *L. punctiforme* as occurring only on petioles and leaf nerves of deciduous species of *Quercus* in Europe. In the north and west of the UK, *L. petiolicola* is common in this habitat, and there are over 100 records of that species throughout Europe and into Georgia (the most western part of Asia) mostly on *Quercus* but also on *Castanea*. [One record on *Sorbus* is probably a misidentification of *L. aucupariae* (Schleich.) Darker.] There are very few records of *L. punctiforme*, all from Europe (Cybertruffle's Robigalia 2013; GBIF 2013). It is possible that these two names represent the same fungus. If so, *L. punctiforme* has priority and would be the correct name.

Lophodermium echinophilum, described from Italy on spines of *Castanea* cupules, may also be a synonym. The paraphyses of these species are not circinate (Tehon 1935). *Lophodermium quercus*, described from India on leaves of the evergreen *Quercus leucotrichophora* A. Camus, has septate ascospores (Cannon & Minter 1986). *Lophodermium yangii*, described from China on leaves of *Cyclobalanopsis glauca* (Thunb.) Oerst., has asci 95–130 µm long. All these species therefore also differ significantly from the present fungus.

Lophodermium agathidis, originally described from leaves of *Agathis australis* (D. Don) Loudon (a conifer) has also been reported from a wide range of other plants including *Castanopsis eyrei* (Lin et al. 2012), apparently an exception to the general patterns described earlier. Developing ascomata of *L. agathidis* have a characteristic pale central band on their surface, the asci are 120–170 µm, and the paraphyses do not have circinate apices. *Lophodermium eucalypti*, which has been recorded on conifers, dicotyledons and monocotyledons, is another apparent exception to that general pattern (Johnston 2001). While it has paraphyses with circinate apices, it does not occur on paler areas of the leaf, nor has it been observed with zone lines or conidiomata. It seems to be an Australasian fungus that has also been recorded, perhaps as an introduction, in North America.

Lophodermium foliicola, a species of Europe and North America, typically associates with members of the *Rosaceae* but has occasionally been recorded from other dicotyledonous families. At least some records on the *Fagaceae* are likely to represent early misidentifications by European mycologists attempting to describe fungi on *Nothofagus* and other plants in the southern hemisphere. Specimens on *Crataegus monogyna* Jacq. from the UK (e.g., IMI 296407) have ascomata that are shorter than 1 mm, asci about 80 µm long, ascospores about 55 µm long, and paraphyses with complex curved and sometimes branched apices. *Lophodermium maculare*, another species of Europe and North America, typically occurs on dead leaves of *Vaccinium* species and has ascomata less than 1 mm long, asci 60–120 µm long, ascospores 55–95 µm long, and paraphyses bent, but not circinate, at the apex. An anamorph has not been observed.

In the protologue, *L. mangatepopense*, a predominantly southern hemisphere species, was described as lacking zone lines and conidiomata. Asci were said to be 100–135 µm long and paraphyses to have clavate, not circinate, apices. *Lophodermium minus* has been observed on a wide range of woody plants, mostly in the southern hemisphere, but with some records from China. Paraphyses are uniformly narrow, sometimes branched at the apex, but not circinate. Asci are 100–130 µm long (Cabarroi & Minter 2005). Although accepted in *Lophodermium* by SpeciesFungorum, *L. minus* was moved to *Terriera* by Johnston (Ortiz-García et al. 2003) and is discussed again under that name later in the present work.

The other possible generalist *Lophodermium* species not yet recorded in association with the *Fagaceae* is *L. mangiferae* Koord. As in *L. agathidis*, its developing ascomata have a characteristic pale band on their surface. Asci are 95–110 µm long and ascospores 70–75 µm long, while paraphyses tips are widened but not circinate. *Lophodermium circinatum* clearly differs from *L. mangiferae* and all other generalist species considered in the foregoing paragraphs.

Terriera nitens Y.R. Lin, **sp. nov.** Figs 8–13

Fungal Name FN570067

Differs from *Terriera illiciicola* by smaller ascomata, a well-developed excipulum, and swollen paraphyses and from *T. minor* by the almost circular to broadly elliptical ascomata, strongly sunken subhymenium, and the presence of conidiomata and frequent zone lines.

Type: China, Anhui, Shitai, Dayan, alt. 1100 m, on leaves of *Cyclobalanopsis myrsinifolia* (Blume) Oerst. (*Fagaceae*), 6 July 2006, T. Zhang & Y.R. Lin 2077 (**Holotype** AAUF 68885).

Etymology: *nitens*, referring to the shiny appearance of ascomata.

Colonies on both sides of leaves, forming irregular, light yellow bleached spots, 4–10 mm diam.

Zone lines frequent, grey-brown to black, thin, surrounding the paler areas.

Conidiomata on both sides of leaves, mostly on the upper side, scattered, occasionally confluent in groups of two or three. In surface view, conidiomata 150–210 µm diam., circular or almost circular, applanate, dark brown in the centre and on the perimeter line of the conidioma, gray-brown or almost concolorous with the substratum surface elsewhere, discharging spores through an apical ostiole. In vertical section, conidiomata subepidermal. Upper wall composed of 3–4 layers of nearly colourless, angular cells 3–5.5 µm diam., the cells around apical ostiole blackish brown. Basal wall 7–12 µm thick, consisting of 2–3 layers of brown to dark brown thick-walled cells forming a textura angularis. Trichogynes 18–30 × 1.5–2 µm, cylindrical, tapering to the apex, 2–4-septate. Conidiogenous cells and conidia not observed.

Ascomata in similar positions to conidiomata on the substratum, crowded in the paler areas. In surface view, ascomata 240–320 × 190–250 µm, suborbicular or broadly elliptical, straight or slightly curved, black, shiny, with a clearly marked outline, strongly raising the surface of the substratum, opening by a single longitudinal split $^{3}/_{4}$–$^{5}/_{6}$ the length of the ascoma. In median vertical section, ascomata subepidermal. Covering stroma 10–16 µm thick near the opening, becoming slightly thinner towards the edge, extending to the basal stroma, composed of textura angularis with dark brown to blackish brown, thick-walled cells 2–3.5 µm diam. Along the edge of the ascomatal opening, there is a 5–8 µm thick, flat extension adjacent to the covering stroma which is comprised of markedly black and brittle tissue with no obvious cellular structure. Basal stroma dark brown, comprised of 2–3 layers of 2.5–5 µm diam., thick-walled angular cells. A layer of textura prismatica 8–17 µm thick, composed of nearly colourless or light brown cells 2.5–4.5 µm diam. occurs between the covering and basal stromata. Excipulum 12–16 µm thick near the opening, becoming slightly thinner towards the base, arising from the inner layer of the basal stroma, consisting of colourless textura porrecta. Subhymenium 5–9 µm thick, strongly concave to form a cup shape, consisting of textura porrecta. Paraphyses colourless, thin-walled, smooth, filiform, aseptate, 95–150 × 1–1.2 µm, sometimes swollen at the apex. Asci ripening sequentially, 85–128 × 4.5–6 µm, narrow cylindrical, somewhat short-stalked, apex round, without circumapical thickening, J–, 8-spored. Ascospores arranged in a fascicle, 68–115 × 0.8–1.2 µm, filiform, colourless, aseptate, round at the apex, slightly tapered towards the acute base, with a thin gelatinous sheath.

Additional specimen examined — **China, Hubei:** Shengnongjia, on leaves of *Cyclobalanopsis pentacycla* (Y.T. Chang) Y.T. Chang ex Y.C. Hsu & H.W. Jen (*Fagaceae*), 10 July 2010, Y.R. Lin & G.J. Jia (AAUF 69308).

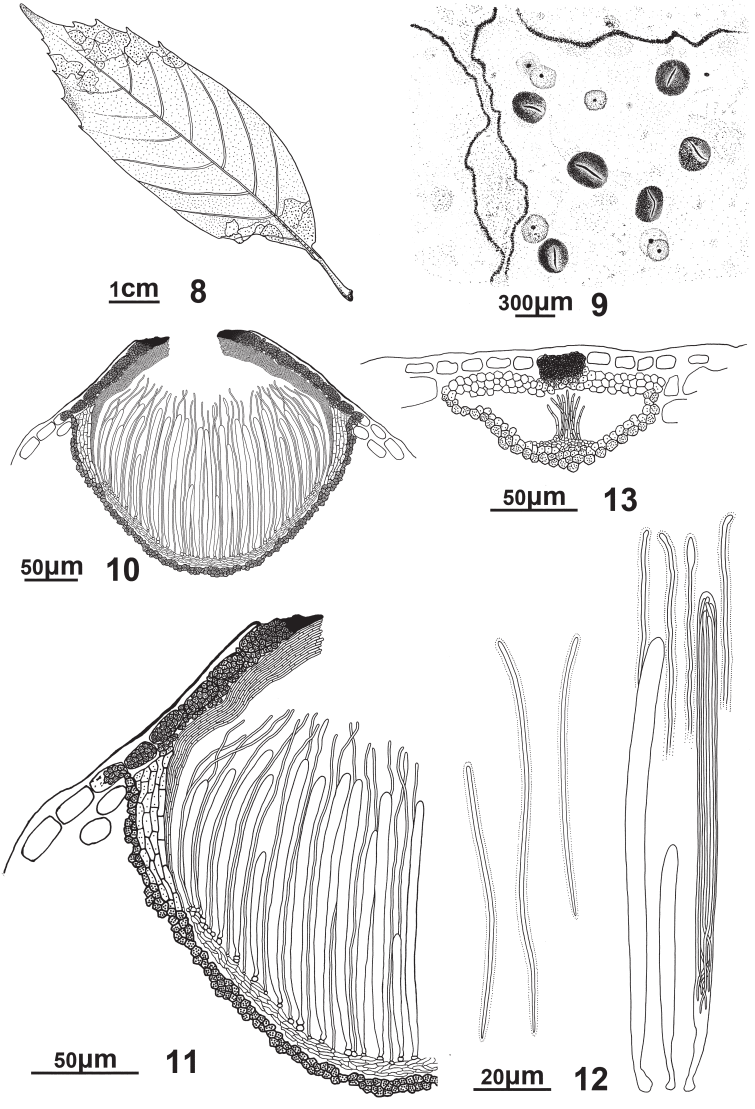
Comments — *Terriera* was established for *T. cladophila* (Lév.) B. Erikss. (Eriksson 1970). IndexFungorum (2013) lists 21 specific and two subspecific

epithets in this genus, of which SpeciesFungorum (2013) accepts 16 species and two varieties in *Terriera*, one epithet in *Lophodermium*, and expresses no opinion about the remaining four epithets, all recent. At least three other species have very recently been described in or transferred to this genus. Most *Terriera* species were originally described in *Lophodermium* or other genera before transfer to *Terriera* by Johnston (Johnston 2001; Ortiz-García et al. 2003). Four *Terriera* species have been recorded associating with trees of the *Fagaceae*: *T. coacervata* Y.R. Lin & Q. Zheng, *T. illiciicola* (S.J. Wang et al.) Q. Zheng & Y.R. Lin, *T. minor* (Tehon) P.R. Johnst., and *T. rotundata* C.L. Hou (Lin et al. 2005, 2012; Song et al. 2012; Zheng et al. 2012).

Terriera coacervata, described on leaves of *Lithocarpus cleistocarpus* (Seemen) Rehder & E.H. Wilson, has ascomata at least 650 (mostly 1000–1800) long, compared with our new species for which the maximum ascomal length is 320 µm. Ascomata of *T. coacervata* tend to coalesce to form complex multiple structures, whereas those of *T. nitens* tend to remain single. There are no records of *T. coacervata* on *Cyclobalanopsis* species.

Terriera illiciicola, described originally on *Illicium verum* Hook. f. (*Schisandraceae*), has also been recorded from the *Fagaceae* on *Lithocarpus cleistocarpus* (Lin et al. 2012). Zheng et al. (2012) reported that *T. illiciicola* does not produce conidiomata, which are abundant in *T. nitens.* Ascomata of *T. illiciicola* are 280–360 × 260–300 µm (Lin et al. 2012) or even larger (300–380 × 280–330 µm; Zheng et al. 2012) while those of *T. nitens* are 240–320 × 190–250 µm so that, despite some overlap, those of *T. illiciicola* are distinctly larger. In comparison with *T. nitens*, the excipulum of *T. illiciicola* is less well developed and paraphyses are not swollen (Zheng et al. 2012). There are no records of *T. illiciicola* on *Cyclobalanopsis* species.

Terriera minor (discussed above under the name *Lophodermium minus*) was originally described from Desecheo Island (Puerto Rico) on *Clusia rosea* Jacq. The holotype is lost, and the species has been neotypified with a collection on *Clusia* sp. from Venezuela (Johnston 1989a). A recent description exists based on *Clusia* specimens from Cuba (Cabarroi & Minter 2005). It differs from *T. nitens* in having no conidiomata in vivo (although conidiomata have been produced in pure culture) and in having more elongated ascomata (0.3–1 mm long), which sometimes coalesce to form more complex structures. When examined in vertical section, the ascoma of *T. minor* has a flat lower wall, a very thin excipulum, and a distinctive flat black area adjacent to both sides of the opening slit, whereas the lower wall in *T. nitens* is strongly curved, the excipulum is thicker, and there is no flat black area adjacent to the opening slit. Paraphyses of *T. minor* are unswollen and sometimes branched, where those of *T. nitens* are often swollen but remain unbranched. There are no records of *T. minor* on *Cyclobalanopsis* species.



Figs 8–13. *Terriera nitens* on *Cyclobalanopsis myrsinifolia*. 8. A leaf bearing zone lines and fruitbodies. 9. Zone lines and fruitbodies observed under a dissecting microscope. 10. Ascoma in median vertical section. 11. Portion of ascoma in median vertical section. 12. Paraphyses, asci, and ascospores. 13. Conidioma in vertical section.

Terriera rotundata was described from Yunnan, China on dead fallen leaves of *Quercus* sp. The protologue stated that conidiomata were not observed while they are present in *T. nitens*. Ascomata are mostly 600–900 µm long and thus much larger than those of *T. nitens*. The two species are similar in having ascomata that are more or less globular in vertical section, but ascospores of *T. nitens* have thin gelatinous sheaths, which have not been observed for *T. rotundata*. There are no records of *T. rotundata* on *Cyclobalanopsis* species. *Terriera nitens* thus differs from all other species of *Terriera* that have been observed on the *Fagaceae*.

Discussion

Lophodermium circinatum is known only from a single collection, and *Terriera nitens* only from two collections augmented by some field observations. As a result, very little can be said with confidence about their biology. The associated plants, *Castanopsis eyrei*, *Cyclobalanopsis myrsinifolia,* and *C. pentacycla* are evergreen trees with leathery leaves that decay slowly. This type of substratum very often carries species of the *Rhytismataceae*. The main leaf fall is in September and October, and by July of the following year these fungi have produced pale areas on the leaf bearing zone lines, conidiomata with trichogynes, conidiogenous cells and conidia (observed only in *L. circinatum* but probably also produced by *T. nitens*), and ascomata with paraphyses, asci, and ascospores. In the case of *T. nitens*, ascomata with fully formed ascospores are also sometimes observed on dead leaves still attached to the tree. Trichogynes are rarely reported for the *Rhytismataceae* (probably because they are easily overlooked), but their presence here suggests the anamorph has a sexual function, something compatible with the few other reports about anamorph biology in the family.

It is likely that colonization of new substrata is effected in humid condition through wind-dispersed ascospores with a mucous sheath, which may help adhesion. There is not enough information to determine which substrata are colonized by the ascospores. It is possible that they infect leaves already destined to senesce and fall in the following autumn, but that strategy is not typical of rhytismataceous species on evergreen leaves. It is perhaps more likely that the ascospores colonize young leaves produced in the spring of that year, while the leaf cuticle is still soft, and then remain in the leaves as a symptomless endobiont.

There is not enough information to determine any possible positive or negative impacts these fungi might have on their associated trees. It is interesting that there are very few collections. This may simply reflect a past lack of attention to this substratum, but other cases are known in the *Rhytismataceae* where a species may be genuinely rare and only very occasionally seen to fruit.

Lophodermium neesii Duby is a good example of this. It occurs in Europe on dead fallen leaves of *Ilex aquifolium* L., a very abundant tree, but has been collected fewer than ten times in 100 years (Minter 2005). One great value of the present work is that it draws attention to the huge diversity of fungi, an important biodiversity resource too often overlooked.

A good place to start when evaluating the conservation status of leaf-inhabiting fungi is to look at the status of the associated plants. *Castanopsis eyrei*, *Cyclobalanopsis myrsinifolia,* and *C. pentacycla* have not yet been evaluated for the IUCN Red List (2013). All three species occur in a range of arboreta, but such collections rarely if ever protect the microfungi associated with plants in their natural environment. *Castanopsis eyrei* is widely distributed in China (GBIF 2013), and there is no immediate reason to suppose this plant is endangered. GBIF (2013) holds very little information about the distribution of *Cyclobalanopsis myrsinifolia*, and no information at all about *C. pentacycla*, and for these two plants it is not possible to comment on their conservation status in the wild. It is also important to evaluate threats to the ecosystems where these fungi were collected. All three collecting sites for these two species are in or close to nature reserves or other conservation areas, and the status of these sites provides some protection, but in each case the reserve or conservation area is only a small remnant of what was once a more extensive ecosystem. There are also new possible threats. One of the three collection sites is in the Shengnongjia region where the impact of big hydro-electric schemes has aroused international attention (Watts 2011). More information about such developments is needed before proper evaluations can be made of threats to fungi in the region. With the present information, and with only one collection of *L. circinatum* and only two of *T. nitens*, the only possible conservation status evaluation for these fungi is, at present, Data Deficient.

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